

## CM 22028 – REINFORCED CLOSTRIDIAL BROTH (as per USP/BP/EP/JP/IP)

### INTENDED USE

For isolation, cultivation and enumeration of Clostridium species, highly nutritive for *Clostridium sporogenes* and other anaerobes.

### PRODUCT SUMMARY AND EXPLANATION

Reinforced Clostridial Broth is an enriched, non-selective medium formulated by Hirsch and Frinsted. This medium was developed for the isolation of spore-forming anaerobes, especially *Clostridium* spp. It conforms to the harmonized USP/EP/JP/BP/IP requirements. This medium can be used for diluting an inoculum of vegetative cells of *Clostridium perfringens* as suggested by Barnes and Ingram. It can also be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese or for enumeration of *Clostridium* species in tube dilution counts. Other spore forming anaerobes, *Streptococci* and *Lactobacilli* also grow in these media.

### COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Beef extract	10.000
Yeast extract	3.000
Glucose monohydrate	5.000
Sodium chloride	5.000
Soluble starch	1.000
Cysteine hydrochloride	0.500
Sodium acetate	3.000
Agar	0.500

### PRINCIPLE

The medium contains peptone and beef extract which acts as the sources of nitrogen, vitamins and amino acids. Yeast extract provides B-complex vitamins. Glucose monohydrate is a complex carbohydrate and sodium chloride maintains the osmotic balance. Soluble starch detoxifies metabolic byproducts. Cysteine hydrochloride is added as a reducing agent and sodium acetate acts as a buffer. The small amount of agar in the broth preparation reduces the diffusion of oxygen through the fluid. The medium can be made selective by addition of 15 – 20 mg Polymyxin B per litre of media.

### INSTRUCTION FOR USE

- Dissolve 38 grams in 1000 ml purified/distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely.
- Dispense into tubes or flasks as desired.
- Sterilize by autoclaving at 15 psi pressure (121° C) for 15 minutes.
- Cool the medium before use.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Dehydrated powder** : Cream to yellow homogeneous free flowing powder



**Appearance of Prepared medium** : Light yellow coloured clear solution in tubes.  
**pH (at 25°C)** : 6.8±0.2

### INTERPRETATION

Culture characteristics observed in an anaerobic atmosphere, after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period 24-48
<i>Clostridium sporogenes</i>	19404	50-100	Good-Luxuriant	30 - 35°C.	Hours 24-48
<i>Clostridium sporogenes</i>	11437	50-100	Good-Luxuriant	30 - 35°C.	Hours 24-48
<i>Bacteroides vulgatus</i>	8482	50-100	Good-Luxuriant	30 - 35°C.	Hours 24-48
<i>Bacteroides fragilis</i>	23745	50-100	Good-Luxuriant	30 - 35°C.	Hours 24-48
<i>Clostridium perfringens</i>	13124	50-100	Good-Luxuriant	30 - 35°C.	Hours

### PACKAGING

In 100 & 500 gm packaging size.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

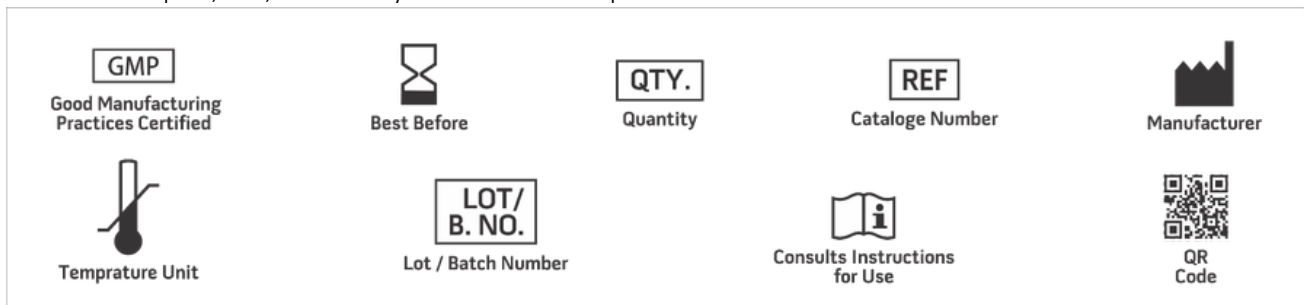
**Product Deterioration:** Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

### DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

### REFERENCES

1. The United States Pharmacopoeia. 2009. Amended Chapters 61, 62 & 111, The United States Pharmacopoeial Convention Inc., Rockville, MD.
2. Directorate for the Quality of Medicines of the Council of Europe (EDQM). 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
3. Japanese Pharmacopoeia. 2008. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.
4. Hirsch, A., and E. Grinstead. 1954. Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J. Dairy Res. 21:101-110.
5. Barnes, Ingram. J Appl Bact. 1956;19.
6. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.
7. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia.



**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For professional use only.**

